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ISOLATION OF ANIMAL VIRUSES FROM WATER

A REPORT OF PRELIMINARY RESEARCH

DIVISION OF RESEARCH

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INTRODUCTION

There have been several reports (1,2) showing that the coliform index may not be a reliable indicator of the presence or absence of enteric viruses. Although infectious hepatitis is the only animal virus reliably confirmed as being transmitted by water, it is possible that other, particularly enteric (poliovirus, Echo, Coxsackie viruses) viruses may be dispersed by this route (3,4). It is known that, in the field, sewage treatment procedures do not always result in an effluent free of enteric viruses (5,6,7) even after chlorination.

To date, there is no method available for the enumeration of enteroviruses in water samples, which is capable of detecting virus at very low levels, and at the same time is relatively inexpensive. A quantitative method, not yet fully tested, uses large numbers of tissue cultures (8) and would probably prove too expensive as a routine procedure. Several qualitative methods exist (9, 10, 11) but they require a great many carefully controlled steps before virus isolation is obtained.

The simplest method thus far described is that of Cliver (personal communication) and it was this method and various modifications of it which were used in this study.

MATERIALS AND METHODS

Viruses

Echo viruses 9 and 11, kindly supplied by Connaught Medical Research Laboratories (CMRL) were employed. The $TCID_{50}$ (tissue-culture-infecting-dose, 50%) on African (Grivet) green monkey kidney cells was about 10^7 infectious units (i.u.) per 0.5 ml. for Echo 11, 10^4 iu/0.5 ml. for Echo 9. The viruses were stored frozen at -20°C until used. Dilution to the required level for experimentation was carried out in maintenance medium.

Medium & Cells

ELY medium (Earles lactalbumen + yeast) with antibiotics and 1% calf serum was used for the maintenance of the Grivet monkey kidney cells (GMK). The GMK cells were obtained in suspension from CMRL and were diluted, as recommended, before seeding, with ELY and 3% calf serum with antibiotics: Tubes were incubated stationary at 36°C, whereas Petri plate cultures were grown at 36°C under 10% CO_2 and high humidity. All cultures were fluid changed to ELY and 2% serum at 5 days.

Experimental

Virus suspension was diluted in tenfold steps and added to 100 ml. samples of distilled water to give about 1, 10^2, 10^3 and 10^4 i.u. per 100 ml.

The water samples were then filtered through a 47 mm diameter, 0.45 μ millipore filter in the usual way. The filter, which according to Cliver adsorbs 90+% of the virus particles, was then treated in one of the following ways, to recover the

virus. In all cases, the presence of virus was detected by observing inoculated cultures for the development of typical cytopathogenic effect (CPE) after incubation at 36°C for three days.

(1) The filter was placed in 4 ml. of phosphate-buffered saline (PBS) or ELY containing 30% fetal calf serum, in a (50 mm diameter) plastic Petri dish. After 30 minutes at room temperature (RT), the eluate was tested for the presence of virus by inoculation on to GMK tube cultures (Cliver's method).

(2) A tissue culture sheet grown in a Petri plate was drained, and the filter was placed face downwards on the sheet. After 30 minutes at room temperature, the filter was removed, maintenance medium added and the plate incubated.

(3) The same procedure as in (2) above, was employed, but the filter was left in the dish upon the addition of maintenance medium. After incubation, the filter is removed before reading the plate.

(4) The filter was placed in about 4 ml. of ELY and 2% calf serum in a Petri dish; after 30 minutes at RT 0.5 ml. of the eluate was inoculated into each of three GMK tissue culture tubes. Maintenance medium was added and the tubes incubated.

RESULTS

Only preliminary work was attempted in this investigation, but the following results serve as an indication of the efficiencies of the various methods. Using Cliver's original method, virus can be detected when the filtered water sample contains virus at 20 - 1000 i.u. per 100 ml. According to Cliver, the elution of the virus is the limiting step and varies in efficiency from 20 - 100% for unknown reasons. The present fairly poor recovery may be the result of a particularly inefficient elutions but the method would appear to be not sufficiently sensitive.

By method (2), virus was detected only at a level when 1000 i.u. were present in the 100 ml. sample; when the filter is left in contact with the cells throughout incubation (method 3) virus could be detected at 100 i.u. per 100 ml. There may be an advantage in a method whereby the filter is incorporated into the culture in some way after the sample has been filtered through it. The filters have little or no effect on the growth of GMK cultures.

The maintenance medium with 2% calf serum, used in method (4) is not an efficient enough fluid to elute virus from the filter at room temperature in 30 minutes, a much higher percentage of serum apparently being necessary.

CONCLUSIONS

None of the methods outlined above, as they stand, appear to be sufficiently sensitive to detect the virus levels expected in even untreated waters, although they would probably be sufficient for raw sewage, or possibly untreated sewage effluents.

However, several possibilities of further modification exist:

(a) Seed or filter tissue culture (TC) cells on top of the filter, after the sample has been filtered; the filter is then placed in a Petri dish and maintenance medium added followed by incubation. The filter can, after a suitable incubation period, be stained to determine whether CPE has developed.

(b) Mix virus-containing sample, electrolyte (to prevent damage to the TC cells) and suspended cells, stir mechanically and filter. Float the filter on to a nutrient medium and incubate. Remove the filter and stain to determine the presence of CPE.

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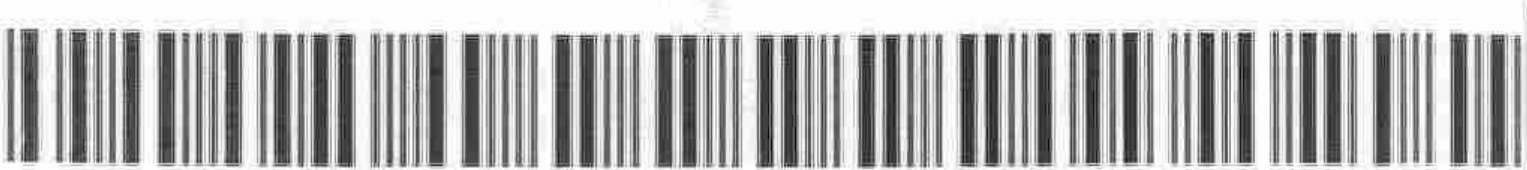
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